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Correlated evolution of fish host length and parasite spore size: a tale from myxosporeans inhabiting elasmobranchs [☆]



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ABSTRACT

Myxozoa represent a diverse group of microscopic cnidarian endoparasites alternating between invertebrate and vertebrate hosts. Of the approximately 2,600 species described predominantly from teleost fish, only 1.8% have been reported from cartilaginous fishes (Elasmobranchii). As ancestral vertebrate hosts of myxozoans, elasmobranchs may have played an important role in myxozoan evolution, however, they are also some of the largest vertebrate hosts known for this group of parasites. We screened 50 elasmobranchs belonging to nine species and seven families, from various geographical areas, for myxozoan infection. We found a 22% overall prevalence of myxozoans in elasmobranchs and describe five species new to science. We investigated, for the first known time, the evolution of spore size within three phylogenetic clades, *Ceratomyxa*, *Sphaerospora* sensu stricto and *Parvicapsula*. We found that spores from elasmobranch-infecting myxozoans were on average $4.8\times$ (*Ceratomyxa*), $2.2\times$ (*Parvicapsula* clade) and $1.8\times$ (*Sphaerospora* sensu stricto except polysporoplasmic *Sphaerospora* spp.) larger than those from teleosts. In all analysed clades, spore size was correlated with phylogenetic position. In ceratomyxids, it was further strongly positively correlated with fish body size and habitat depth, independent of cellular composition of the spores and phylogenetic position in the tree. While in macroparasites a host size-correlated increase in parasite size occurs on a large scale and is often related to improved exploitation of host resources, in microscopic parasites size ranges vary at the scale of a few micrometres, disproportionate to the available additional space in a large host. We discuss the ecological role of these changes with regard to transmission under high pressure and an invertebrate fauna that is adapted to deeper marine habitats.

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1. Introduction

Elasmobranchs tend to be much larger than teleost fishes. The most massive teleost, the bump-head sunfish (*Mola alexandrini*) at 2.3 tonnes is almost an order of magnitude smaller than the largest elasmobranch fish, the whale shark (*Rhincodon typus*) of approximately 20.6 tonnes and 20 m long (Freedman and Noakes, 2002). Maximum length is available for tens of thousands

of taxa (Froese and Pauly, 2020, www.fishbase.org, accessed 5 June 2020) with 90% of teleosts measuring only between 3 and 83 cm while 90% of elasmobranchs (sharks, rays and skates) grow from 36 to 403 cm (Fig. 1). Apart from their large size, elasmobranchs are interesting from an evolutionary perspective as they are phylogenetically distinct, ancient vertebrate groups, with almost 1,200 species (Stein et al., 2018) which are mostly marine and distributed all over the world.

Although elasmobranchs are parasitised by a multitude of protist and metazoan parasites, relatively little information is available on myxozoans (Cnidaria: Myxozoa). This diverse group of cnidarian endoparasites has a complex life cycle, which likely arose by the incorporation of a vertebrate (mainly fish) into simple life

[☆] Note: New sequence data reported in this paper are available in GenBank under accession numbers MW396732 and MW396733.

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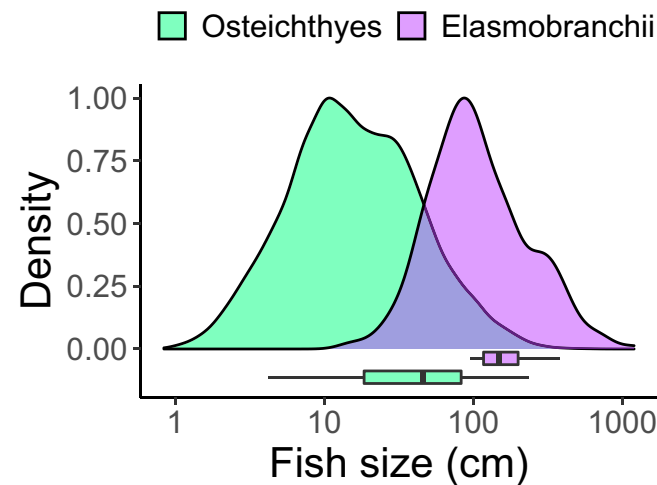


Fig. 1. Distribution of fish host body size of Elasmobranchii (purple) and Osteichthyes (green). Elasmobranchs measure around 145 cm on average while bony fish grow to around 26 cm on average. Variability in size of host species with myxozoan infections (*Ceratomyxa*, *Parvicapsula* and *Sphaerospora* sensu stricto clades) recorded in the present study is represented by boxplots.

cycles involving only aquatic invertebrate hosts (annelids or bryozoans) (Holzer et al., 2018; Lisnerová et al., 2020). Currently, there are some 2,600 myxozoan species distributed into two classes: Myxosporidia, including the majority of known species; and Malacosporea, with fewer than 20 known species. Diversity of myxozoans seems to be strongly underestimated (Hartikainen et al., 2016; Okamura et al., 2018). This appears to be true also for myxozoans from chimaeras and elasmobranchs (Gleeson and Adlard, 2012; Kodádková et al., 2015; Lisnerová et al., 2020) as only 1.8% of all myxozoan species have so far been reported from elasmobranchs (Lisnerová et al., 2020), with 45 myxozoan species belonging to seven genera (*Ceratomyxa*, *Chloromyxum*, *Ellipsomyxa*, *Kudoa*, *Myxidium*, *Sinuolinea*, and *Sphaerospora*) formally described to date. In elasmobranchs, myxozoans show a worldwide geographic distribution, although the majority of studies are from Australian waters (Gleeson et al., 2010; Gleeson and Adlard, 2011, 2012), the Atlantic coast of Europe (Fiala, 2006; Rocha et al., 2014) and the Atlantic coast of the Americas (Azevedo et al., 2009; Cantatore et al., 2018; Lisnerová et al., 2020).

The study of elasmobranch myxosporidians can give us a better understanding of the ancient evolutionary history of this parasite group and host-parasite co-evolutionary patterns (Fiala, 2006; Kodádková et al., 2015; Lisnerová et al., 2020). Myxozoans from elasmobranchs cluster in six independent lineages, which are frequently placed at the base of teleost-infecting clades (Fiala and Dyková, 2004; Fiala, 2006; Azevedo et al., 2009; Gleeson and

Adlard, 2011, 2012; Rocha et al., 2014; Cantatore et al., 2018; Lisnerová et al., 2020). *Ceratomyxa* represents one of the most comprehensively sampled marine genera and includes 278 described species (Eiras, 2006; Eiras et al., 2018; 111 DNA GenBank records at 12 May 2020) with parasite ssrDNA sequences available from both, teleost and elasmobranch hosts encompassing a variety of host total lengths, ranging from 100 to 380 cm in elasmobranchs and from 4.2 to 200 cm in teleosts (included in Fig. 1). Moreover, ceratomyxids from elasmobranchs do not cluster basally within ceratomyxids (Lisnerová et al., 2020).

Host body size is considered one of the most important factors determining parasite abundance, parasite body size and species richness (Poulin, 2007; Kamiya et al., 2014), but such character correlations have not previously been studied for myxozoans. Spores, which are responsible for parasite dispersal and host invasion (Alexander et al., 2015), contain most characters used in myxozoan taxonomy, with spore size and shape being key for systematics (Lom and Dyková, 2006). Myxospores harbour polar capsules, which are homologous to nematocysts of free-living cnidarians (Americus et al., 2020) and essential for host attachment and invasion. Myxozoan spore morphology frequently contradicts phylogenetic clustering of species (Fiala, 2006; Liu et al., 2019), rendering the majority of genera paraphyletic/polyphyletic, and suggesting a host or environmental impact on spore morphology.

During our recent investigations into myxozoans from chondrichthyan hosts (Lisnerová et al., 2020), we noticed that several parasite species in elasmobranchs develop unusually large spores, encouraging investigations into the evolution of size-related patterns. To shine a light on characters prone to morphological change, and physical and ecological factors associated with modifications in myxozoan spore size and morphology throughout evolution, the present study aimed at describing myxospore morphology in large-bodied fish groups (≥ 1 m), as well as undertaking comparative analyses to determine if morphological and morphometric differences of myxozoans from the same phylogenetic clade correlate with phylogeny, host body size or host habitat depth. We address whether the spore/body size correlation between myxozoans and their hosts is adaptive and discuss the physical and biological challenges for transmission in meso- and bathypelagic zones of the oceans versus shallower water habitats, in relation to myxospore morphological characters, for the first time in this group of parasites.

2. Materials and methods

2.1. Sample collection and parasite morphology

In total, 50 elasmobranch specimens belonging to nine species of seven families were collected off South Africa ($n = 15$), off Argentina ($n = 28$), in the Gulf of Mexico ($n = 6$), and off South Carolina,

Table 1
Elasmobranch hosts sampled during the present study with information on host species, number of individuals and locality.

Host species	Number of individuals	Date of collection	Locality
<i>Haploblepharus edwardsii</i>	1	7 October 2018	Hermanus, SA
<i>Haploblepharus fuscus</i>	2	5 October 2018	Hermanus, SA
<i>Haploblepharus pictus</i>	4	4–11 October 2018	Hermanus, SA
<i>Mustelus mustelus</i>	6	8–11 October 2018	Hermanus, SA
<i>Myliobatis goodei</i>	5	15 December 2014	off the coast of Buenos Aires Province, ARG
<i>Rhizoprionodon terraenovae</i>	1	20 May 2011	Cape Romain Harbor, South Carolina, USA
<i>Rostoraja alba</i>	2	2019	Hermanus, SA
<i>Sphyrna tiburo</i>	6		Gulf of Mexico, USA
<i>Squalus acanthias</i>	23	2018	Off the coast of Buenos Aires Province, ARG
Total number	50		

SA, South Africa; ARG, Argentina; USA, United States of America.

USA ($n = 1$), between 2011 and 2018 (Table 1). Argentinian elasmobranchs were obtained as by-catch by commercial trawlers operating off the coast of Buenos Aires Province. *Rhizoprionodon terraenovae* was captured in the Charleston Harbor region, South Carolina, USA. At both sites, animals were captured following the terms and conditions of local or national regulations. South African elasmobranch specimens were collected from various sampling sites off the coast of Hermanus, Western Cape, South Africa, by longline, handline or by handfishing. Permits for the collection of sharks were issued by the South African Department of Agriculture, Forestry and Fisheries (Permit nos.: RES2018-58 and RES2019-61 issued to the South African Shark Conservancy, and RES2019-105 issued to BCS (Bjoern C. Schaeffner). Gulf of Mexico bonnetheads were collected using trotline in local waters off Sarasota, Florida, USA, under Special Activities License (SAL) research collecting permits issued by the Florida Fish and Wildlife Conservation Commission, USA. Valid names of host species follow the Catalog of Fishes (Eschmeyer et al., 2021, <http://researcharchive.calacademy.org/research/ichthyology/catalog/fishcatmain.asp>, accessed 30 January 2021).

Based on previous experience demonstrating the presence of myxozoans in the biliary and urinary systems of elasmobranchs (Gleeson and Adlard, 2012; Cantatore et al., 2018; Lisnerová et al., 2020), kidneys and gall bladders ($n = 50$) were primarily screened, while liver samples ($n = 21$), muscle ($n = 7$) and gills ($n = 6$) were examined only in a few specimens. Light microscopy was used to detect myxozoan infections. Digital pictures of myxozoan spores and plasmodia from fresh tissues were documented at 400 $\times$ and 1,000 $\times$ magnification using an Olympus BX51 microscope equipped with an Olympus DP70 camera (Olympus, Japan). Measurements (in micrometres), which followed the guidelines of Lom and Arthur (1989) and Heiniger et al. (2008) were obtained from digital images of 20–30 (in one case only seven) spores using ImageJ 1.53e (Schneider et al., 2012) and are presented in species descriptions as the mean $\pm$ S.D., with the range in parentheses.

2.2. ssrDNA amplification

All obtained elasmobranch tissue samples (microscopically positive and negative) were stored in 400 μ L of TNES urea buffer (10 mM Tris-HCl, pH 8, 125 mM NaCl, 10 mM EDTA, 0.5% SDS and 4 M urea) or in 96% ethanol. DNA was extracted using a phenol–chloroform protocol (Holzer et al., 2004) with overnight digestion using proteinase K (50 μ g mL⁻¹, Serva, Germany) at 55 °C.

All (even microscopically negative) samples were PCR-screened for myxozoan infections. Initial PCRs were carried out with primer combinations 18e + 18R (first run) and MyxGP2F + Act1R (second run) using the PCR composition given by Holzer et al. (2004) and PCR cycling parameters following the TITANIUM polymerase protocol of Patra et al. (2018). Partial ssrDNA sequences of myxozoans from positive samples were afterwards obtained by various combinations of general eukaryotic/myxozoan clade-specific primer sets (see Supplementary Table S1).

PCR amplicons were extracted from 1% agarose gels using the Gel/PCR DNA Fragments Extraction Kit (Geneaid Biotech Ltd., USA) and sequenced directly (Seqme, Czech Republic). PCR products with low DNA concentrations or unclear chromatograms were cloned into the pDrive Vector with a Qiagen PCR Cloning Kit (Qiagen, Germany) and transformed into *Escherichia coli* competent cells. Plasmid DNA was extracted using a High Pure Plasmid Isolation Kit (Roche Applied Science, Germany) and 2–5 colonies were sequenced (Seqme, Czech Republic). All sequences obtained in this study were submitted to GenBank at the National Center for Biotechnology Information (NCBI, USA). Four ssrDNA sequences were obtained in a previous study (Lisnerová et al., 2020), where

morphological descriptions were, however, missing. These corresponding records were cross-referenced.

2.3. Phylogenetic analyses

Four datasets were created for phylogenetic analyses: (i) a full myxozoan dataset showing the position of all lineages from elasmobranchs, i.e. ssrDNA dataset of 138 sequences; (ii) a *Ceratomyxa*-ssrDNA dataset composed of 111 sequences of *Ceratomyxa* spp.; (iii) a *Sphaerospora* ssrDNA dataset composed of 34 sequences of *Sphaerospora* sensu stricto spp. (including polysporoplasmic sphaerosporids as they form part of this monophyletic clade) and (iv) a *Parvicapsula* ssrDNA dataset composed of 17 sequences of *Parvicapsula* spp., *Gadimyxa* spp. and *Sphaerospora testicularis*. The sequences were aligned in MAFFT v6.864b (Katoh et al., 2005) using the E-INS-i algorithm implemented in Geneious Prime 2019.0.4 (Kearse et al., 2012) and manually edited by removal of ambiguously aligned regions.

Maximum likelihood (ML) analyses were performed in RAxML v7.0.3 (Stamatakis, 2006) implemented in Geneious Prime 2019.0.4 using the GTR + Γ model which was selected as the best-fitting model of evolution in jModelTest (Posada, 2008). Bootstraps were calculated from 1,000 replicates. Bayesian inference (BI) analysis was performed in MrBayes v3.0 (Ronquist and Huelsenbeck, 2003) implemented in Geneious Prime 2019.0.4 with the GTR + Γ model of evolution. Posterior probabilities were calculated over 1 million generations via two independent runs of four simultaneous Markov Chain Monte Carlo algorithms with every 100th tree saved and burn-in set to 10% (100,000 generations).

2.4. Relationships between spore characteristics and host/environmental parameters

Data for the maximum body length of Elasmobranchii and Osteichthyes species were retrieved from FishBase (Froese and Pauly, 2020, www.fishbase.org, accessed 5 June 2020) using the function popchar in the rfishbase package (Boettiger et al., 2012) and visualized by means of kernel density distribution function. Variability in size of host species with myxozoan infections recorded in the present study (*Ceratomyxa*, *Parvicapsula* and *Sphaerospora* sensu stricto clades) was visualized by boxplots in Fig. 1.

For parasite morphometry associations, the following parameters were considered: myxospore length, myxospore width or thickness, section area (two-dimensional (2D) representation of spore), polar capsule length, polar capsule width, and cellular composition of sporoplasms. The myxopore section area was estimated in ImageJ 1.53e (Schneider et al., 2012) from proportional 2D line drawings of spores, which were depicted according to myxospore dimensions given in original species descriptions (Supplementary Tables S2–S4). Based on the significant correlation of myxospore length, thickness, and section area in *Ceratomyxa* (Supplementary Fig. S1), for downstream analyses investigating the relationship between spore size and host/ecological features, myxospore section area was used as a proxy for size.

With regard to host parameters, body sizes of the fish host species were represented by maximum total lengths of each species while host habitat depths were represented by the maximum depths recorded, both according to FishBase (Froese and Pauly, 2020, www.fishbase.org, accessed 5 June 2020). Information on maximum fish host body size, maximum host habitat depth and the myxospore size of each species is listed in Supplementary Tables S2–S4.

We used three different datasets encompassing the members of (i) the *Sphaerospora* sensu stricto clade (34 records, 30 species, myxospore section area 23.6–246.8 μ m², host size 6.2–234.0 cm, host habitat depth 12–1,460 m, 1–12 sporoplasms), (ii) the *Parvi-*

capsula clade (17 records, 16 species, myxospore section area 31.6–145.5 μm^2 , host size 22.0–203.0 cm and host habitat depth 80–3,000 m) and (iii) the *Ceratomyxa* clade (112 records, 98 species, myxospore section area 33.8–979.8 μm^2 , host size 4.2–380.0 cm and host habitat depth 1–4,800 m). The latter dataset represents the most comprehensive one and is characterised by a large range of host sizes and habitat depths in both teleost and elasmobranch hosts. It was thus used for all analyses, while the remainder of the datasets were only analysed where statistical analyses were possible (see Section 3 for details).

To determine if morphological character changes in myxozoan spores can be associated with phylogenetic position and/or host (host size) and ecological features (host habitat depth), different approaches were used. To determine if spore size is a proxy for the evolutionary age of a species and if phylogeny has an influence on myxospore size, we calculated phylogenetic signal using Pagel's λ in the phytools package (Revell, 2012). Thereafter, we performed ancestral spore size reconstruction and mapped host and parasite traits to the tree using the fastAnc and dotTree functions in phytools. To characterise the relationship between polar capsule length and myxospore size characteristics (length and thickness of the spore, myxospore section area) and to estimate if host size and host habitat depth are good predictors of myxospore section area, we used phylogenetic generalized linear models (pGLS) in the caper package (Orme et al., 2013, <https://cran.r-project.org/web/packages/caper/index.html>). We used pGLS models to eliminate the influence of phylogenetic signal on the studied relationships. The analyses were performed separately for all studied parasite groups. In the case of *Sphaerospora*, spores can have one to several sporoplasms, potentially occupying more space and hence influencing spore size. Thus, we also included sporoplasm composition in the analysis of this group. Furthermore, linear regression was used to analyse the relationship between fish host body size and fish host habitat depth (to observe if the results of the two previous analyses may be affected by the fact that fish size depended on fish habitat depth). For all size-related analyses, response (myxospore size attributes) and quantitative explanatory variables (host body size, host habitat depth, sporoplasm number) were logarithmically transformed prior to the analyses to avoid highly positively skewed distribution of the data. All analyses were conducted using the R software (R Core Team, 2020, <https://www.R-project.org>) and visualised using phytools and ggplot2 (Wickham, 2011) packages. The level of statistical significance is expressed as *P* value. The *P* value threshold for statistical significance was 0.05.

2.5. Data accessibility

R code, alignments and tables with details about hosts and parasites used in analyses are available at MendeleyData via the link: <https://doi.org/10.17632/xjv5ymk8zs.1#file-39bcedbd-cf40-4700-ba41-b60c48a97816>.

3. Results

3.1. Uncharted diversity of myxosporeans in elasmobranch fishes

Spore-forming plasmodia or mature spores were found in 56% (5/9) of elasmobranch species, representing 22% of examined specimens (11/50). Infections were encountered in gall bladders (10%; 5/50), kidneys (12%; 6/50) and muscle (14%; 1/7), while livers (0/21) and gills (0/6) did not contain any myxozoan parasites, even when screened by PCR. Mature myxospores (and rarely also plasmodia) of coelozoic myxosporeans belonging to *Ceratomyxa* and *Sphaerospora*, which are formally described in this study (Table 2),

were observed in three sharks (*Mustelus mustelus*, *Rhizoprionodon terraenovae*, *Squalus acanthias*) and one ray (*Myliobatis goodei*) (Fig. 2A–N). Spores of *Ceratomyxa* sp. (Fig. 2T) and *Parvicapsula* sp. (Fig. 2O–Q) were observed in the bile of *Sphyrna tiburo* and in the kidney of *S. tiburo*, respectively, but we do not describe them as new species due to an insufficient amount of morphological data. Spores of a histozoic *Kudoa* sp. (Fig. 2R, S) matching the original morphological description and published ssrDNA sequence data of *K. hemiscylli* were found in the muscle of *S. tiburo* (Table 2).

3.2. Taxonomic descriptions

In this study, we provide formal species descriptions of five myxosporean parasites of elasmobranch hosts based on the morphological and molecular (ssrDNA) sequence differences from published species.

3.2.1. Taxonomic description of *Ceratomyxa* spp.

Phylum Cnidaria Hatschek, 1888

Subphylum Myxozoa Grassé, 1970

Class Myxosporea Bütschli, 1881

Order Bivalvulida Schulman, 1959

Family Ceratomyxidae Doflein, 1899

Genus *Ceratomyxa* Thélohan, 1892

***Ceratomyxa sigmoidea* Lisnerová n. sp. (Fig. 2A–C)**

Type host: *Mustelus mustelus* (L.), common smooth-hound (Carcharhiniformes: Triakidae), adult specimen collected in October 2018.

Type locality: Rietfontein, Hermanus, South Africa (34° 26' 332" S, 19° 13' 486" E).

Other localities: Vermont, Hermanus, South Africa (34° 26' 210" S, 19° 13' 663" E).

Site of infection: Coelozoic in gallbladder (myxospores floating in the bile).

Prevalence of infection: Overall: 67% (4/6); Vermont, Hermanus, South Africa: 100% (1/1); Rietfontein, Hermanus, South Africa: 60% (3/5).

Etymology: Refers to the Greek word sigmoidis, which means S-shaped, with one spore valve curving upwards and the other one curving downwards.

ZooBank registration: urn:lsid:zoobank.org:act:9A1481B1-D2F0-430D-B424-86FC6E8B1C17.

Molecular data (two host specimens): Identical partial ssrDNA sequences (1,384 bp and 1,093 bp; expected total gene length around 2,000 bp) obtained from two host specimens.

Material deposited: DNA material stored at the Protistological Collection of the Institute of Parasitology, Biology Centre, Czech Academy of Sciences, České Budějovice (Acc. number IPCAS Prot 62), ssrDNA sequence (1,384 bp) deposited under the GenBank Acc. Number MW396732.

Description of sporogonic stages: Plasmodia not observed.

Description of myxospores (three host specimens): Mature spores of arcuate shape, typically with one valve curved upwards and other one curved downwards. Spore length 11.1 ± 0.6 (10.3–12.6) μm and thickness 8.4 ± 5.4 (77.2–96.8) μm ($n = 26$; type host); spherical polar capsules, length 4.8 ± 0.5 (4.0–5.9) μm and width 4.8 ± 0.5 (4.1–6.0) μm ($n = 26$; type host), 6–7 polar filament coils ($n = 2$, type host). Length of suture position 42.1 ± 4.7 (35.0–56.0) μm ($n = 26$; type host). Sutural line straight. Posterior angle convex 123.4 – 173.2° ($n = 20$, type host). Non-significantly different measurements of spores and polar capsules from other two host specimens given in Supplementary Table S5. One binucleate sporoplasm.

Remarks: In total, 15 *Ceratomyxa* species have so far been described from elasmobranchs and most of them seem to be strictly host-specific (Lisnerová et al., 2020) (Table 2). *Ceratomyxa*

Table 2
Comparison of newly described species with related myxosporeans.

Myxozoan species	Fish host	ssrDNA GenBank Acc. No.	Spore dimensions (length × width/thickness in μm)	Reference
<i>Ceratomyxa sigmoidea</i> n. sp.	<i>Mustelus mustelus</i>	MW396732	SP 11.1 ± 0.6 (10.3–12.6) × 84.4 ± 5.4 (77.2–96.8); PC 4.8 ± 0.5 (4.0–5.9) × 4.8 ± 0.5 (4.1–6.0)	Present study
<i>Ceratomyxa barbini</i> n. sp.	<i>Myliobatis goodei</i>	MK937839	SP 11.7 ± 1.3 (9.5–13.8) × 32.9 ± 8.1 (20.0–44.9); PC 3.7 ± 0.4 (3.0–4.4) × 3.7 ± 0.3 (3.0–4.2)	Lisnerová et al. (2020); Present study
<i>Ceratomyxa</i> sp. 2 ex <i>Sphyrna tiburo</i>	<i>Sphyrna tiburo</i>	MK937838	SP 6.9 ± 0.0 (6.9–6.9) × 48.9 ± 2.7 (46.2–51.5); PC 2.7 ± 0.1 (2.6–2.8) × 2.8 ± 0.1 (2.7–3)	Lisnerová et al. (2020); Present study
<i>Ceratomyxa abbreviata</i> Davis, 1917	<i>Rhizoprionodon terraenovae</i>	NA	SP 14 × 17; PC 4.5 × 4.5	Davis (1917)
<i>Ceratomyxa agilis</i> Thélohan, 1892	<i>Dasyatis pastinaca</i>	NA	SP 6–7 × 11–12	Thélohan (1892)
<i>Ceratomyxa attenuata</i> Davis, 1917	<i>Rhizoprionodon terraenovae</i>	NA	SP 9 × 115; PC 4.5 in diameter	Davis (1917)
<i>Ceratomyxa carcharhini</i> Gleeson et Adlard, 2011	<i>Carcharhinus melanopterus</i>	JF911815–16	SP 10.0 ± 0.7 (9–11.5) × 58.1 ± 7.1 (46–77.5); PC 3.8 ± 0.2 (3.5–4) × 3.7 ± 0.2 (3.5–4)	Gleeson and Adlard (2011)
<i>Ceratomyxa flagellifera</i> Davis, 1917	<i>Carcharhinus</i> sp.	NA	SP 12 × 118; PC 6 in diameter	Davis (1917)
<i>Ceratomyxa jamesoni</i> Kudo, 1933 (<i>C. taenia</i> Jameson, 1931)	<i>Triakis semifasciata</i>	NA	SP 7.5–9.5 × 95–117	Jameson (1931), Kudo (1933)
<i>Ceratomyxa lunata</i> Davis, 1917	<i>Galeocerdo cuvier</i>	NA	SP 7–9 × 15–30; PC 4 in diameter.	Davis (1917)
<i>Ceratomyxa melanopteri</i> Gleeson et Adlard, 2011	<i>Carcharhinus melanopterus</i>	JF911817	SP 11.3 ± 1.2 (10–13.5) × 44.5 ± 9.3 (28–58.5); PC 3.9 ± 0.1 (3.5–4) × 4.0 ± 0.2 (3.5–4.5)	Gleeson and Adlard (2011)
<i>Ceratomyxa mesospora</i> Thélohan, 1892	<i>Sphyrna zygaena</i> ; <i>Sphyrna tiburo</i>	NA	SP 8 × 50–60; PC 4.5 in diameter	Thélohan (1892)
<i>Ceratomyxa negaprioni</i> Gleeson et Adlard, 2011	<i>Negaprion acutidens</i>	JF911816	SP 11.1 ± 0.7 (10–12.5) × 27.7 ± 2.7 (20.5–33); PC 5 ± 0.4 (3.5–5.5) × 4.9 ± 0.3 (3.5–5.5)	Gleeson and Adlard (2011)
<i>Ceratomyxa recurvata</i> Davis, 1917	<i>Sphyrna zygaena</i>	NA	SP 8–9 × 16; PC 4.5 in diameter	Davis (1917)
<i>Ceratomyxa sphaerulosa</i> Thélohan, 1892	<i>Mustelus canis</i> ; <i>Galeorhinus galeus</i>	NA	SP 10–12 × 90–100; PC 6–7 × 5	Thélohan (1892)
<i>Ceratomyxa sphairophora</i> Davis, 1917	<i>Rhizoprionodon terraenovae</i>	NA	SP 12 × 115–149; PC 6 in diameter	Davis (1917)
<i>Ceratomyxa taenia</i> Davis, 1917	<i>Rhizoprionodon terraenovae</i>	NA	SP 6 × 140–150; PC 3 in diameter	Davis (1917)
<i>Ceratomyxa scissura</i> (<i>Leptotheca scissura</i>) Davis, 1917	<i>Dasyatis hastata</i> ; <i>Gymnura altavela</i>	NA	SP 11 × 22	Davis (1917)
<i>Kudoa hemiscylli</i> Gleeson, Bennett et Adlard, 2010	<i>Sphyrna tiburo</i>	NA	SP 11.0 ± 0.3 (10.6–11.6) (spore apical width) × 9.3 ± 0.4 (8.5–9.6) (spore apical thickness) × 10.6 ± 0.4 (10.1–11.0) (spore lateral width) × 7.8 ± 0.1 (7.8–7.9) (spore length)	Lisnerová et al. (2020); Present study
<i>Kudoa hemiscylli</i> Gleeson, Bennett et Adlard, 2010	<i>Hemiscyllium ocellatum</i>	GU324949	SP 9.9 ± 0.43 (9.1–10.8) (spore apical width) × 9.5 ± 0.38 (8.7–10.1) (spore apical thickness) × 9.6 ± 0.36 (9.1–10.5) (spore lateral width) × 7.6 ± 0.35 (7–8.2) (spore length)	Gleeson et al. (2010)
<i>Kudoa carcharhini</i> Gleeson, Bennett et Adlard, 2010	<i>Carcharhinus caudatus</i>	GU324968	SP 10.1 ± 0.49 (9.5–11.2) (spore apical width) × 9.8 ± 0.6 (7.8–10.6) (spore apical thickness) × 9.8 ± 0.49 (8.5–10.6) (spore lateral width) × 8.2 ± 0.56 (7.2–9.5) (spore length)	Gleeson et al. (2010)
<i>Parvicapsula</i> sp. ex <i>Sphyrna tiburo</i>	<i>Sphyrna tiburo</i>	MK937852	SP 11.3 ± 0.4 (10.7–11.6) × 13.3 ± 0.0 (13.3–13.3) × 11.9 (thickness); PC 4.0 ± 0.2 (3.8–4.2) × 3.9 ± 0.2 (3.8–4.1)	Lisnerová et al. (2020); Present study
<i>Sphaerospora alata</i> n. sp.	<i>Mustelus mustelus</i>	MW396733	SP 10.4 ± 0.7 (9.0–11.9) × 11.5 ± 0.8 (10.2–12.9); PC 3.8 ± 0.4 (2.9–4.5) × 3.7 ± 0.4 (2.9–4.2)	Present study
<i>Sphaerospora terraenovae</i> n. sp.	<i>Rhizoprionodon terraenovae</i>	MK937855	SP 11.0 ± 0.8 (10.0–12.2) × 13.6 ± 0.8 (13.0–15.2); PC 4.0 ± 0.2 (3.6–4.3) × 3.8 ± 0.2 (3.8–4.2)	Lisnerová et al. (2020); Present study
<i>Sphaerospora argentinensis</i> n. sp.	<i>Squalus acanthias</i>	MK937853	SP 11.7 ± 1.1 (10.3–13.7) × 13.4 ± 2 (7.2–16.3); PC 4.5 ± 0.3 (4.1–5.1) × 4.4 ± 0.3 (3.6–4.8)	Lisnerová et al. (2020); Present study
<i>Sphaerospora araii</i> Arthur et Lom, 1985	<i>Beringraja rhina</i> as <i>Raja rhina</i>	NA	SP 14.3–18.4 × 14.8–18.5	Arthur and Lom (1985)

SP, spore; PC, polar capsule; NA, data not available.

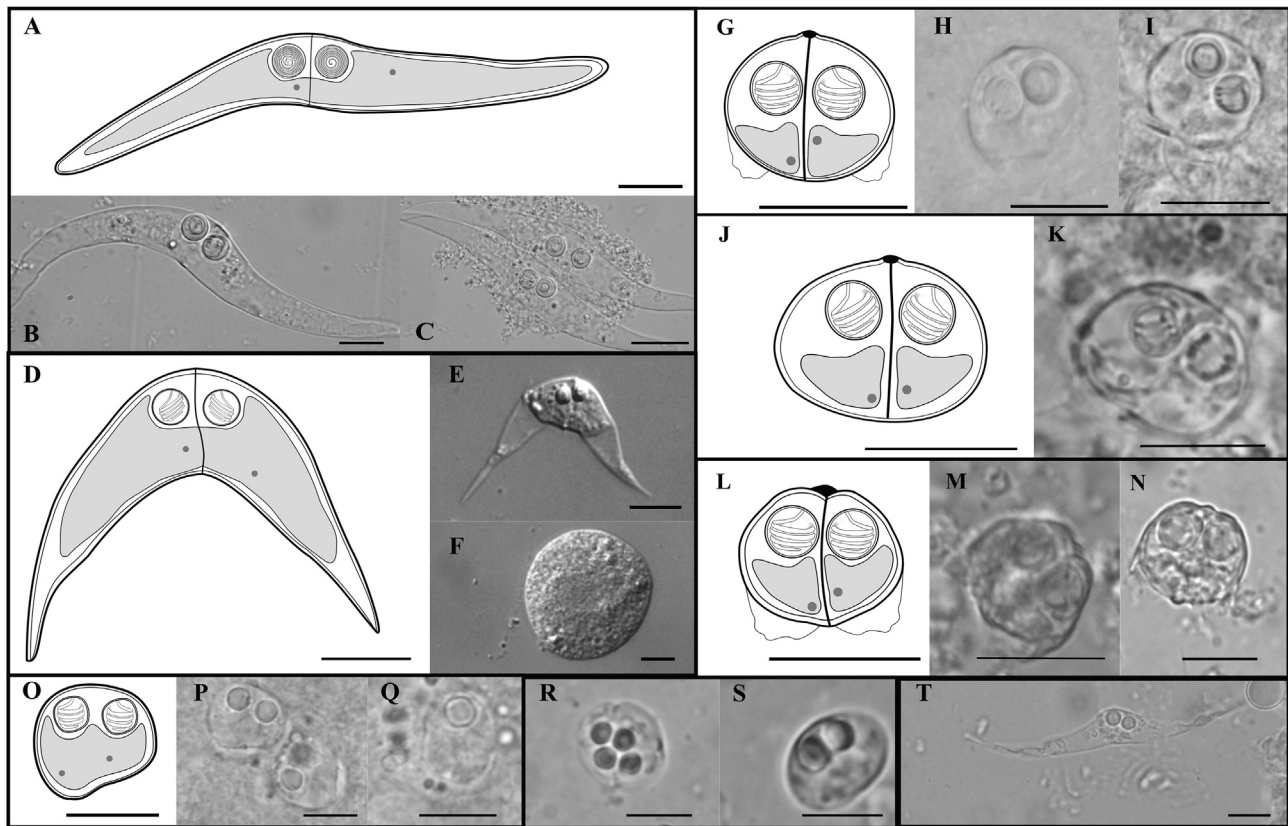


Fig. 2. Drawings and light microscopy pictures of newly described species in this study. *Ceratomyxa sigmoidea* n. sp. (A) Line drawing, (B) mature spore in frontal view and (C) spore doublet after the rupture of disporoblast; *Ceratomyxa barbini* n. sp. (D) Line drawing, (E) mature spore in frontal view, (F) round-shaped plasmodium; *Sphaerospora alata* n. sp. (G) Line drawing, (H) mature spore in frontal view with posterior protuberances and (I) mature spore with focus on polar filament turns; *Sphaerospora terraenovae* n. sp. (J) Line drawing, (K) mature spore in frontal view; *Sphaerospora argentinensis* n. sp. (L) Line drawing, (M and N) mature spores in frontal view with posterior protuberances; *Parvicapsula* sp. ex *Sphyrna tiburo*. (O) Line drawing, (P) mature spores in frontal view, (Q) mature spore in side view; *Kudoa hemiscylli*. (R) Mature spore in apical view and (S) in side view; mature spore of *Ceratomyxa* sp. ex *Sphyrna tiburo* (T). Scale bars = 10 µm.

sigmoidea n. sp. is distinguishable from its shark- and ray-inhabiting congeners by the unique spore shape (one end curved upwards, one downwards), larger dimensions (generally longer and thicker spores, except for *C. sphaerulosa*, *C. sphairophora* and *C. taenia*, which are thicker), and by host species (shark *M. mustelus* vs. teleost fishes) (Table 2).

***Ceratomyxa barbini* Cantatore et Lisnerová n. sp. (Fig. 2D–F)**

Type host: *Myliobatis goodei* (Garman, 1885), Southern eagle ray (Myliobatiformes: Myliobatidae), adult specimen collected in December 2014.

Type locality: Off the coast of Buenos Aires Province, Argentina (34°–42° S, 53°–62° W).

Site of sporogonic development: Coelozoic in gallbladder (plasmodia and myxospores floating in the bile).

Prevalence of infection: 20% (1/5).

Etiology: In honor of the Argentinian ichthyologist Santiago Barbini who has made many contributions to the knowledge of the biology, ecology and diversity of elasmobranchs in the Argentine Sea.

ZooBank registration: urn:lsid:zoobank.org:act:43D91159-5A66-43B6-8B4C-10DEB050AD08. **Molecular data** (single host specimen): Partial ssrDNA sequence (1,647 bp; expected total gene length around 2,000 bp) obtained from a single host specimen refers to *Ceratomyxa* sp. 1 MG6 in Lisnerová et al. (2020).

Material deposited: DNA material and slides with spores stored at the Protistological Collection of the Institute of Parasitology, Biology Centre, Czech Academy of Sciences, České Budějovice (Acc. number IPCAS Prot 61), ssrDNA sequence deposited under the GenBank Acc. Number MK937839.

Description of sporogonic stages (single host specimen): Disporic plasmodia, highly polymorphic of spherical, oval or irregular shape with variable dimensions, 19.1 ± 8.9 (6.6–36.5) µm maximum length ($n = 10$) (Fig. 2F).

Description of myxospores (single host specimen): Mature spores of arcuate shape with pointed valves; spore length 11.7 ± 1.3 (9.5–13.8) µm and thickness 32.9 ± 8.1 (20.0–44.9) µm ($n = 20$; type host); polar capsules spherical, equal in size, length 3.7 ± 0.4 (3.0–4.4) µm and width 3.7 ± 0.3 (3.0–4.2) µm ($n = 20$); 4–5 coils in each polar filament ($n = 2$). Valves roughly equal. Length of suture position 16.8 ± 3.9 (10.0–22.5) µm. Sutural line straight. Posterior angle convex (72.6–166.4°). One binucleate sporoplasm.

Remarks: *Ceratomyxa barbini* n. sp. overlaps by spore dimensions with the elasmobranch-infecting species *C. negaprioni* and *C. melanopteri*. However, they differ by valve shape (pointed vs. round) and ssrDNA sequence data in which *C. barbini* n. sp. matches the sequence of *C. negaprioni* by 90.5% (131 nt differences across 1350 bp) and *C. melanopteri* by 90.5% (131 nt differences across 1337 bp). The newly described species can be distinguished from other *Ceratomyxa* spp. reported from elasmobranchs by unique spore shape/dimensions, ssrDNA sequences and host species (Table 2).

3.2.2. Taxonomic description of *Sphaerospora* spp.

Phylum Cnidaria Hatschek, 1888

Subphylum Myxozoa Grassé, 1970

Class Myxosporea Bütschli, 1881

Order Bivalvulida Schulman, 1959

Family Sphaerosporidae Davis, 1917

Genus *Sphaerospora* Thélohan, 1892

***Sphaerospora alata* Lisnerová n. sp. (Fig. 2G–I)**

Type host: *Mustelus mustelus* (L.), common smooth-hound (Carcharhiniformes: Triakidae), adult specimen collected in October 2018.

Type locality: Vermont, Hermanus, South Africa (34° 26' 210" S, 19° 13' 663" E).

Other locality: Rietfontein, Hermanus, South Africa (34° 26' 332" S, 19° 13' 486" E).

Site of infection: Coelozoic in kidney renal tubules.

Prevalence of infection: Overall: 50% (3/6); Vermont, Hermanus, South Africa: 100% (1/1); Rietfontein, Hermanus, South Africa: 40% (2/5).

Etymology: Refers to the Latin word “ala” which means wing and corresponds to small wing-like postero-lateral protuberances on the mature spore.

ZooBank registration: urn:lsid:zoobank.org:act:59C73DDB-9F8B-40E0-B5F6-02D285811E52.

Molecular data (single host specimen): Partial ssrDNA sequence (1,058 bp; expected total gene length around 2,600 bp) obtained from a single host individual.

Material deposited: DNA material stored at the Protistological Collection of the Institute of Parasitology, Biology Centre, Czech Academy of Sciences, České Budějovice (Acc. number IPCAS Prot 63), ssrDNA sequence deposited under the GenBank Acc. Number MW396733.

Description of sporogonic stages (single host specimen): Disporic pseudoplasmodia of oval shape ranging 25.4–28.6 µm ($n = 1$).

Description of myxospores (three host specimens): Mature spores wider than long, pointed apically in frontal view; length 10.4 ± 0.7 (9.0–11.9) µm and thickness 11.5 ± 0.8 (10.2–12.9) µm; polar capsules equally-sized, almost spherical, length 3.8 ± 0.4 (2.9–4.5) µm and width 3.7 ± 0.4 (2.9–4.2) µm ($n = 30$; type host); each polar filament with 4–5 coils ($n = 12$; type host). Non-significantly different measurements of spores and polar capsules for other two host specimens given in [Supplementary Table S5](#). Spore surface without striations but containing small postero-lateral protuberances. Two uninucleate sporoplasms.

Remarks: Only one sphaerosporid species has so far been described from an elasmobranch host (*Sphaerospora araii* Lom et Arthur, 1985) which has much larger spores and infects a different host species (sphaerosporids are usually strictly host-specific; [Patra et al., 2018](#)) than *S. alata* n. sp. Unfortunately, no ssrDNA sequence data is available for *S. araii* for comparison.

***Sphaerospora terraenovae* Lisnerová n. sp. (Fig. 2J–K)**

Type host: *Rhizoprionodon terraenovae* (Richardson, 1836), Atlantic sharpnose shark (Carcharhiniformes: Carcharhinidae), adult specimen collected in May 2011.

Type locality: Charleston Harbor region, South Carolina, United States of America (33° 03' 528" N, 79° 21' 268" W).

Site of sporogonic development: Coelozoic in kidney renal tubules.

Prevalence of infection: 100% (1/1).

Etymology: Refers to the type-host species *Rhizoprionodon terraenovae*.

ZooBank registration: urn:lsid:zoobank.org:act:8A69A053-F40D-4773-BC5C-76BA6165D881.

Molecular data (single host specimen): Partial ssDNA sequence (1,214 bp; expected total gene around 2,600 bp) refers to *Sphaerospora* sp. 3 RT_2 of [Lisnerová et al. \(2020\)](#).

Material deposited: DNA material stored at the Protistological Collection of the Institute of Parasitology, Biology Centre, Czech Academy of Sciences, České Budějovice (Acc. number IPCAS Prot 65), rDNA sequence deposited under the GenBank Acc. Number MK937855.

Description of sporogonic stages (single host specimen): Disporic pseudoplasmodia of round, oval or irregular shape ranging 20.6–27.0 µm in diameter ($n = 4$).

Description of myxospores (single host specimen): Mature spores wider than long, with pointed apical end in frontal view, length 10.0–12.2 µm and thickness 13.0–15.2 µm; polar capsules subspherical, equally sized, length 3.6–4.2 µm and width 3.8–4.2 µm ($n = 7$); 4–5 polar filament coils in each polar capsule ($n = 2$). Spore without any striations, filaments or protuberances. Two uninucleate sporoplasms.

Remarks: The only sphaerosporid species that has so far been described from an elasmobranch host (*S. araii*) has much longer spores and a different host than the newly described *S. terraenovae* n. sp. *Sphaerospora terraenovae* n. sp. can be distinguished from *S. alata* n. sp. by the lack of postero-lateral protuberances on the mature spore, the larger size of mature spores, by a different host species, and by ssrDNA sequences (similarity 98.2%; 20 nt differences across 1058 bp).

***Sphaerospora argentinensis* Cantatore et Lisnerová n. sp. (Fig. 2L–N)**

Type host: *Squalus acanthias* (L.), Picked dogfish (Squaliformes: Squalidae), adult specimen collected in 2018.

Type locality: Off the coast of Buenos Aires Province, Argentina (34°–42° S, 53°–62° W).

Site of sporogonic development: Coelozoic in kidney renal tubules.

Prevalence of infection: 8.7% (2/23).

Etymology: Refers to the country of origin, Argentina.

ZooBank registration: urn:lsid:zoobank.org:pub:FC429562-47E1-44D6-8B4A-473D9AE682EE.

Molecular data (two host specimens): Identical partial ssDNA sequences (2,213 and 2,526 bp; expected total gene length around 2,600 bp) obtained from two host specimens refers to *Sphaerospora* sp. 1 SA in [Lisnerová et al. \(2020\)](#).

Material deposited: DNA material and slides with spores stored at the Protistological Collection of the Institute of Parasitology, Biology Centre, Czech Academy of Sciences, České Budějovice (Acc. number IPCAS Prot 64), ssrDNA sequence (2,526 bp) deposited under the GenBank Acc. Number MK937853.

Description of sporogonic stages (type host): Disporic pseudoplasmodia of round to oval shape ranging 21.7–27.5 µm ($n = 3$).

Description of myxospores (type host): Mature spores subspherical, length 11.7 ± 1.1 (10.3–13.7) µm and thickness 13.4 ± 2.0 (7.2–16.3) µm. Two equally-sized spherical polar capsules, length 4.5 ± 0.3 (4.1–5.1) µm and width 4.4 ± 0.3 (3.6–4.8) µm ($n = 20$). Polar filaments with 4 coils ($n = 1$). Valves with 4 longitudinal striations and postero-lateral bulges on each valve. Sutural line straight and prominent. Two uninucleate sporoplasms.

Remarks: *Sphaerospora argentinensis* n. sp. can be distinguished from *S. araii* which has much longer spores ([Table 2](#)). *Sphaerospora argentinensis* n. sp. can be distinguished from *S. terraenovae* n. sp. and *S. alata* n. sp. by different spore sizes, smaller postero-lateral protuberances than those of *S. alata* n. sp. and by host species ([Table 2](#)).

3.2.3. Details about *Parvicapsula* sp. (Fig. 2O–Q)

Type host: *Sphyrna tiburo* (L.), Bonnethead (Carcharhiniformes: Sphyrnidae).

Type locality: off coast of Florida, USA, Gulf of Mexico, United States of America.

Site of infection: Coelozoic in kidney renal tubules.

Prevalence of infection: 17% (1/6).

Molecular data: Partial ssDNA sequence (1,433 bp; MK937852; expected total gene length around 2,000 bp) obtained from single host individual refers to *Parvicapsula* sp. 1 ST_4 of [Lisnerová et al. \(2020\)](#).

Description of sporogonic stages: Plasmodia not observed.

Description of myxospores (single host specimen): Mature spores asymmetric, wider than long, length 12.6 (11.6–13.5) μm , width 13.4 (13.2–13.5) μm and thickness 8.6 μm ; relatively large equally-sized polar capsules, almost spherical in shape, length 4.0 (3.8–4.2) μm and width 3.9 (3.8–4.1) μm ($n = 2$); number of polar filament coils not determined. Spore surface smooth. Single distinct binucleate sporoplasm.

3.3. Elasmobranch-infecting myxozoans create unique host group-defined phylogenetic branches

The species described in the present study clustered in two out of four major myxozoan lineages, i) the polychaete-infecting lineage harbouring the members of the *Ceratomyxa* and *Parvicapsula* clades, and ii) the *Sphaerospora* sensu stricto clade accommodating most sphaerosporids (Fig. 3). Ceratomyxids from elasmobranchs formed a strongly supported subclade of the *Ceratomyxa* clade (ML/BI = 99/1; Fig. 3) containing only one species, *C. informis*, from a teleost host. This subclade emerged more recently than some of the teleost-infecting lineages, and its branches are comparatively short, indicating limited divergence of ssrDNA sequences. In contrast to *Ceratomyxa*, the shark-infecting *Parvicapsula* sp. ex *Sphyrna tiburo* created the most basal lineage of the whole *Parvicapsula* clade with maximum nodal support in our analyses (ML/BI = 100/1; Fig. 3). All three newly described sphaerosporids, *S. argentinensis* n. sp., *S. alata* n. sp. and *S. terraenovae* n. sp. clustered together with another shark-infecting *Sphaerospora* sp. ex *Mustelus schmitti* (ML/BI = 100/1; Fig. 3), as a separate elasmobranch-infecting group within the *Sphaerospora* sensu stricto clade. This subclade was positioned at the base of the two subclades, one composing species from freshwater + marine fish and the other one species from tetrapods. Another subclade of sphaerosporids infecting marine teleosts clustered more basal to the elasmobranch-infecting species. These findings are in line with previous studies (most recently Cantatore et al., 2018; Lisnerová et al., 2020).

3.4. Spore size is dependent on phylogeny and elasmobranch myxozoans have larger spores than those from teleosts

Spore section area was related to phylogeny in all studied groups (*Sphaerospora* sensu stricto: $\lambda = 0.808$, $P < 0.001$; *Parvicapsula*: $\lambda = 0.741$, $P = 0.010$; *Ceratomyxa*: $\lambda = 0.999$; $P < 0.001$) indicating that spore size does not change sporadically but that an increase/decrease in spore size is characteristic to particular subclades. Myxozoan spores from elasmobranchs were generally larger in size than those of teleosts (Figs. 4A, B, 5).

In *Sphaerospora* sensu stricto, the largest myxospores belonged to polysporoplasmic *Sphaerospora* spp., commonly found in teleosts (*S. sparis*, *S. motemariini*, *Sphaerospora* sp. ex *Liza ramada*, *Sphaerospora* sp. ex *Chelon labrosus*; myxospore section area 154–247 μm^2 ; estimated ancestral value of 163 μm^2). These are sphaerosporids whose spores contain multiple instead of characteristically one or two sporoplasms (Bartošová et al., 2013). Shark-infecting sphaerosporids were on average 1.8 $\times$ larger than classical sphaerosporids but 0.6 $\times$ smaller than polysporoplasmic *Sphaerospora* spp. The four smallest sphaerosporids with a myxospore section area of 24–29 μm^2 (*S. hankai*, *S. gutta*, *S. diversa*, *S. angulata*) were obtained from different teleosts in freshwater habitats. The largest polysporoplasmic *Sphaerospora* sp., *S. motemariini*, was 10.5 $\times$ larger than the smallest one with two sporoplasms, *S. hankai*. Ancestral myxospore section area values were estimated as 97 μm^2 for shark-infecting sphaerosporids, 66 μm^2 for frog-infecting sphaerosporids, 85 μm^2 for freshwater fish-infecting sphaerosporids, 66 μm^2 for marine fish-infecting sphaerosporids

and 74 μm^2 for *Sphaerospora* sensu stricto as a whole (details in Fig. 4A and Supplementary Table S2).

Parvicapsula sp. ex *S. tiburo* had the second largest spore (section area of 127 μm^2) of all *Parvicapsula* clade members and was only 0.9 $\times$ smaller than *Sphaerospora testicularis*, a morphologically unusual member of the clade. However, spores of *Parvicapsula* sp. ex *S. tiburo* were on average 2.2 $\times$ larger than all other parvicapsulids. *Gadimyxa atlantica* was the smallest parvicapsulid with a section area of 32 μm^2 . The ancestral myxospore section area value was estimated as 100 μm^2 for the *Parvicapsula* clade (Details in Fig. 4B and Supplementary Table S3).

In the most species-rich group, *Ceratomyxa*, members of the elasmobranch-infecting clade (including *C. informis* as the only teleost-infecting species) had a 4.4 $\times$ larger myxospore section area than species of teleost-infecting clades. This group contained the four largest *Ceratomyxa* spp. in our dataset (*Ceratomyxa* sp. ex *Scyliorhinus canicula*, *C. sigmoidea* n. sp., *Ceratomyxa* sp. ex *Sphyrna tiburo*, and *C. melanopteri*), with myxospore section areas ranging from 512 to 980 μm^2 (ancestral myxospore section area 349 μm^2 ; Fig. 5, Supplementary Table S4). The four smallest *Ceratomyxa* spp. with a myxospore section area between 3 and 41 μm^2 (*Ceratomyxa cardinalis* ex *Ostorhinchus cyanosoma*, *C. moseri*, *C. heronensis*, *C. bohari*) were found in small reef fishes (Fig. 5, Supplementary Table S4; largest subclade). The largest *Ceratomyxa* sp. (*Ceratomyxa* sp. ex *Scyliorhinus canicula*) has a myxospore section area that is 29.0 $\times$ larger than the one of the smallest species (*C. cardinalis*). The ancestral myxospore section area of the whole *Ceratomyxa* group was estimated as 187 μm^2 , while most *Ceratomyxa* teleost-infecting subclades were estimated at section areas around 100–200 μm^2 , with some exceptions for instance in the clade of *C. anko*, *C. appendiculata* and *C. pantherini* (more details in Fig. 5 and Supplementary Table S4). Increased spore dimensions in ceratomyxids were strong predictors of increased polar capsule length (myxospore length – $F_{1,110} = 320.6$; $P < 0.001$; myxospore thickness – $F_{1,110} = 44.92$; $P < 0.001$; myxospore section area – $F_{1,110} = 126.57$; $P < 0.001$) (Fig. 6A).

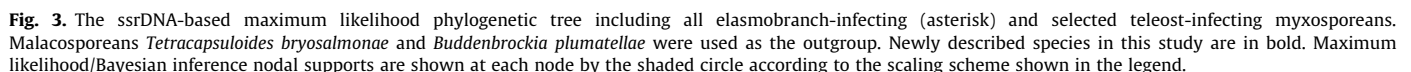
3.5. The largest ceratomyxids inhabit large-bodied fishes from mesial and bathyal habitats

We tested the influence of host size and the environmental factor of habitat depth on spore size (myxospore section area), performing phylogenetic correction, and showed that only the spore size of *Ceratomyxa* is influenced by host size ($F_{1,104} = 335.07$; $P < 0.001$) and habitat depth ($F_{1,103} = 108.06$; $P < 0.001$) (Fig. 6B), while the sizes of *Parvicapsula* and *Sphaerospora* sensu stricto clade members were not significantly influenced by host size ($F_{1,14} = 1.01$; $P = 0.33$; $F_{1,30} = 0.23$, $P = 0.64$), habitat depth ($F_{1,13} = 0.07$; $P = 0.79$; $F_{1,28} = 3.01$, $P = 0.09$) or the number of sporoplasms ($F_{1,29} = 0.06$; $P = 0.81$; *Sphaerospora*).

We further found that larger fish in our database inhabit deeper waters ($t_{104} = 7.426$; $P < 0.001$; Fig. 6C).

4. Discussion

This study provides insights into the diversity, morphology and phylogeny of myxozoan parasites encountered in a large variety of elasmobranch hosts. Elasmobranchs are an ancestral host group for the Myxozoa (Kodádková et al., 2015; Holzer et al., 2018), however, they have so far been poorly explored as myxozoan hosts. We provide morphological descriptions and ssrDNA sequences for new species of myxozoans from large-bodied fish, thereby providing unique datasets for phylogenetic and morphometric analyses. We noticed that myxozoans from sharks and rays occupy separate



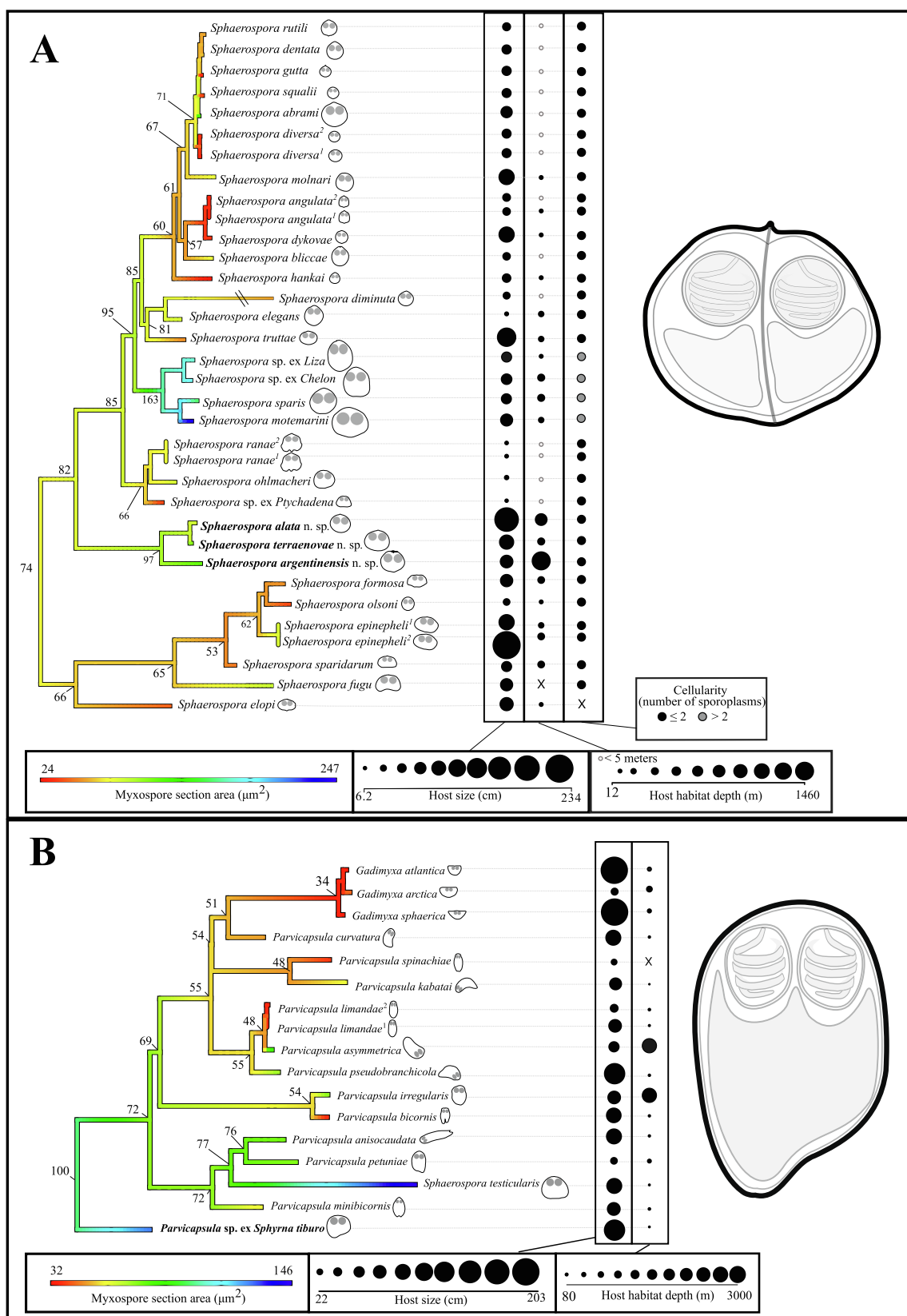


Fig. 4. The *ssrDNA*-based maximum likelihood phylogenetic trees with myxospore shape, host habitat depth, host size, cellularity (in the case of *Sphaerospora* sensu stricto) and estimation of ancestral myxospore section area for important nodes, mapped to the tree. The tree heat map refers to spore size (myxospore section area). Elasmobranch-infecting species are marked in bold. Data not available for a given species are marked with X. (A) *Sphaerospora* sensu stricto clade; (B) *Parvicapsula* clade.

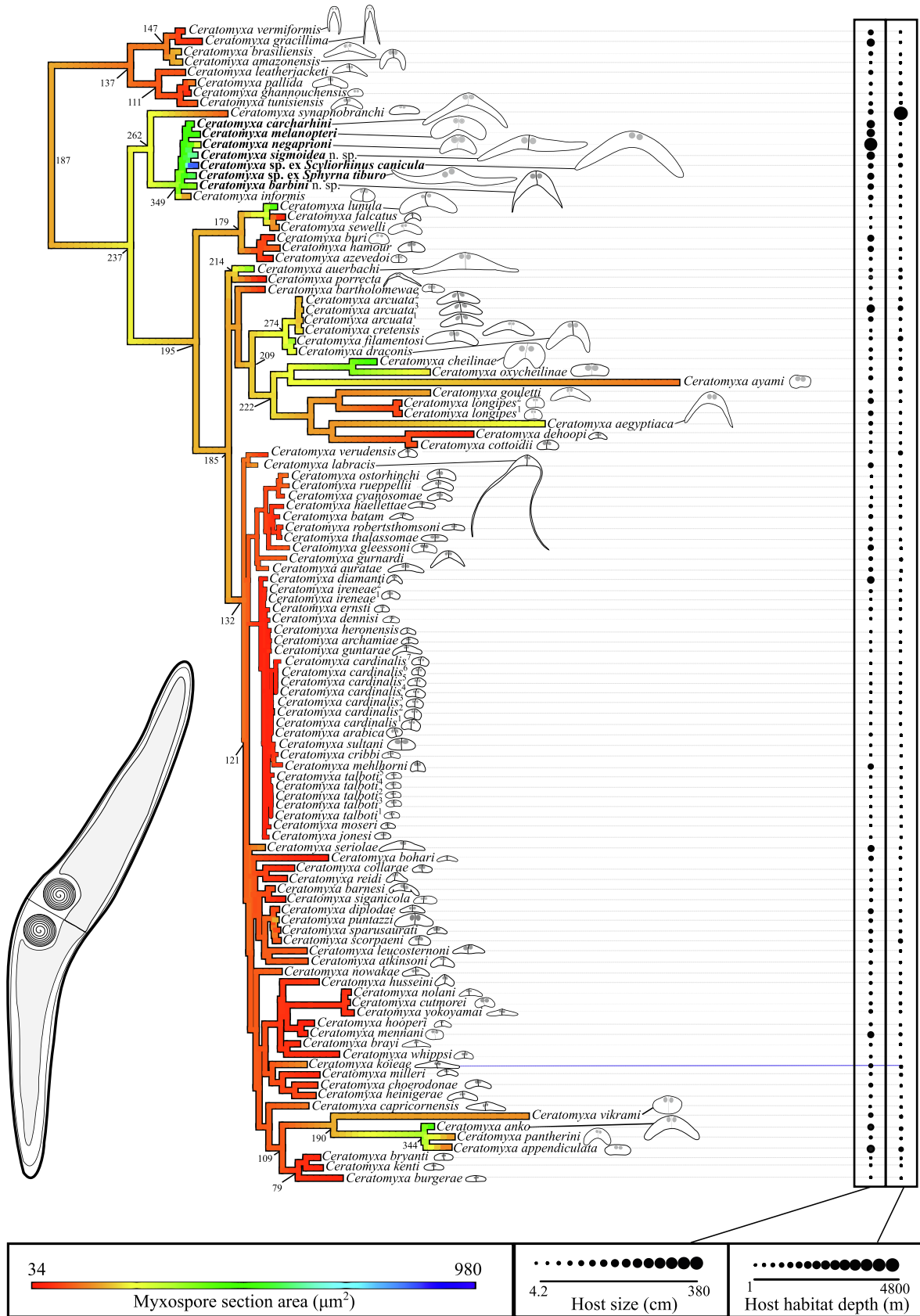


Fig. 5. The ssrDNA-based maximum likelihood phylogenetic tree of the *Ceratomyxa* clade with myxospore shape, host habitat depth, host size, and estimation of ancestral myxospore section area for important nodes, mapped to the tree. Tree heat map refers to spore size (myxospore section area). Elasmobranch-infecting species are marked in bold.

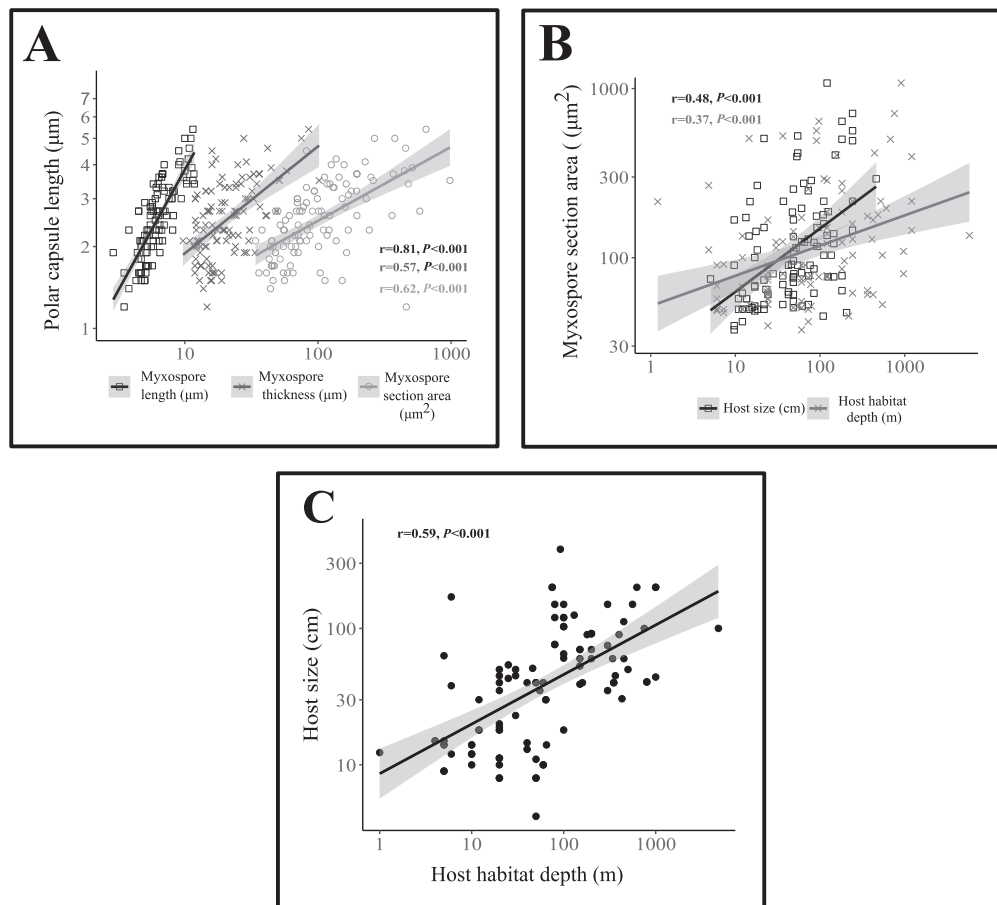


Fig. 6. Relationship between host-defined parameters and myxospores of *Ceratomyxa* spp. Generalized linear models with phylogenetic correction of (A) average length of polar capsules and average myxospore length/thickness/myxospore section area; (B) myxospore section area and maximum fish body size/host habitat depth; (C) maximum fish body size and maximum fish habitat depth. The P value threshold for statistical significance was 0.05.

phylogenetic clades and that their spores are considerably larger than those obtained from most of their teleost-inhabiting congeners. Comprehensive statistical methods and evolutionary models were used to analyse spore size-related patterns in three phylogenetic clades, with particular focus on the species-rich *Ceratomyxa* clade. Although cellularity and basic spore shapes of myxozoans in small and large fish hosts are generally the same (with the exception some of *Sphaerospora* spp.), remarkable differences in myxospore section area were observed in different clades, especially in *Ceratomyxa* spp., where size incrementation was almost five-fold. Size-related correlations in parvicapsulids and *Sphaerospora* sensu stricto showed limited significance, likely due to small sample size. However, in the comprehensive *Ceratomyxa* dataset, myxospore section area was strongly correlated with both, host body size and habitat depth. In contrast to parvicapsulids and ceratomyxids, sphaerosporids are not largest in elasmobranchs. Indeed, the largest spores are found among the marine teleost-infecting *Sphaerospora* spp. harbouring multiple sporoplasms (i.e., polysporoplasmic *Sphaerospora* spp., Sitjà-Bobadilla and Alvarez-Pellitero, 1995), with cellularity having a more significant impact on myxospore section area than host size. This can be explained by the extreme reduction of these parasites to only a few cells, with every additional cell causing a significant size change.

As described previously (Gleeson and Adlard, 2011; Lisnerová et al., 2020), *Ceratomyxa* spp. from ancient elasmobranch hosts do not cluster in the most basal position. Their positioning may be explained by an initial invasion of teleost intermediate hosts and a later switch to elasmobranchs (Lisnerová et al., 2020), or

an origin in ancient Chondrichthyes and a switch by movement of teleosts into freshwater habitats (Zatti et al., 2017) by the first ceratomyxids. However, it allows us to conclude that spore size changes are adaptive and large size is not an ancestral condition.

Various biological factors have so far been recognized to act on parasite size with host body size being one of the most obvious host-related factors (Poulin, 2007). The positive correlation of large parasites in large-bodied hosts, coined as Harrison's rule (Harrison, 1915), may be associated with more efficient usage of available niche space by parasite individuals in larger hosts (Sasal et al., 1999). In fact, larger hosts have larger organs which represent larger habitats that provide more space and nutrients for parasites to exploit (Kuris et al., 1980; Poulin, 1995). Examples of Harrison's rule have been documented for a wide spectrum of other parasites and their hosts, e. g. wing lice and birds (Johnson et al., 2005), different insect species and their hosts (Kirk, 1991), digenean flukes and their fish hosts (Sasal et al., 1999) and rhizocephalan parasites and crustaceans (Poulin and Hamilton, 1997). However, as myxozoans are truly microscopic parasites and spore size changes are effective at some tens of micrometres it is unclear whether the size of the host body, which varies at a much larger scale, plays a crucial role in the evolution of spore size variations, when compared with large-bodied metazoan parasites (Kirk, 1991; Poulin and Hamilton, 1997; Sasal et al., 1999; Johnson et al., 2005).

It is important to relate changes in spore morphology not only to the host in which these spores are produced but also to the habitat for their dispersion and to their target hosts. The size of parasite developmental stages in an intermediate host can be associated

with larger body size in the final host (Fredensborg and Poulin, 2005; Benesh and Hafer, 2012). Ceratomyxid myxospores infect polychaete invertebrates as final hosts (Køie et al., 2008), in which a different morphotype of spore is produced that re-infects the fish host, whereby the typical dixenous myxozoan life cycle is completed. Polychaetes represent a major component of macrobenthic assemblages, in terms of abundance, diversity and biomass, at every bathymetric level (Gambi and Giangrande, 1986; Abbiati et al., 1987; Baldrighi et al., 2013), with many large species, e. g. the sulfid worm *Paralvinella sulfincola* (up to 7 cm) or the giant tube worm (*Riftia pachyptila*, up to 3 m), occurring in deep sea habitats. Whether larger myxospores are required to infect large polychaetes is unclear, since very few life cycles have been elucidated in polychaetes (only nine are known to date; Atkinson et al., 2019) and the data are too scarce to discover any trends.

Interestingly, large elasmobranch-infecting *Ceratomyxa* spp. inhabit fishes that grow to greater sizes and inhabit deeper oceanic habitats (75–750 m, Smith and Brown, 2002; this study), likely due to selective pressures exerted by environmental conditions (Poulin and Hamilton, 1995). The predominance of large-sized organisms at greater depth, defined as the 'bigger-deeper phenomenon', has been described in different groups of marine organisms such as e.g., crustaceans (Ardizzone et al., 1990), cephalopods (Roper and Boss, 1982), free-living cnidarians (Matsumoto et al., 2003; Daly, 2006) or fish (Massuti et al., 1996). The deep sea is the largest ecosystem on Earth but organisms living there must contend with high pressure, low temperature, decreased dissolved oxygen levels, food scarcity and scarcely available or irregularly distributed hosts. However, hardly anything is known about myxozoans inhabiting the bathyal (>1,000 m) or abyssal (>5,000 m) zones (Noble, 1966; Yoshino and Noble, 1973; Lom et al., 1975; Threlfall and Khan, 1990). In these habitats, environmental parameters themselves may be responsible for a larger spore size as higher pressure causes increased water density and hence spores may have to be larger (heavier relative to surface area) to sink and reach benthic polychaete hosts at all. Our parasite size-depth correlations are based on the maximum depth reported for the relevant host species and for more meaningful outcomes, future analysis of spore sizes along depth gradients should focus on single species in non-migratory hosts occurring at a variety of depths, such as *C. arcuata* or *C. appendiculata* in the angler, *Lophius piscatorius*, which can be found between 20 and 1,000 m.

In parasitic crustaceans, body size tends to increase with both water depth and latitude, environmental correlates of temperature (Poulin, 1995; Poulin and Hamilton, 1995). Comparing sister taxa, those inhabiting higher latitudes or deeper water tend to be larger-bodied than their relatives in warmer waters, independent of other variables such as host type or size. This complies with Bergmann's rule that states that within a broadly distributed taxonomic clade, populations of species of larger size are found in colder environments and offers a further interpretation of our data from myxozoans. Similar patterns can be found in monogeneans (Poulin, 1996) and sea lice (Samsing et al., 2016), while opposite trends are often observed in non-aquatic hosts (e.g. Dallas et al., 2019). These examples illustrate that responses of parasite body size to selective environmental pressures such as temperature or depth can vary and, in myxozoans, spore size may further be influenced by infection requirements in the invertebrate host. However, few studies failed to find an association between host size and parasite size (Poulin, 2007), and the present data strongly indicates a size correlation for several myxozoan genera and their fish hosts. The fact that spores of larger size appear in isolated clades characterized only by large hosts may additionally suggest that adaptive constraints limit the range that myxozoans can use, as has been demonstrated in lice (Clayton et al., 2003). Size may thus reinforce cospeciation by preventing parasites from switching to hosts of

smaller size, despite the generally extraordinary capacity for host switching in this parasite group, which is associated with an extremely high evolutionary diversification rate (Holzer et al., 2018).

This is the first time that size-related models of evolution and host-associated changes were elucidated in myxozoans. We believe that we have confirmed Harrison's rule for myxozoans by a positive correlation between fish host size and myxozoan spore size, however, other effects such as depth, water pressure and invertebrate target host can at present not be excluded as factors contributing to the observed size variation. The adaptive basis of the observed phenomenon remains poorly understood and requires further investigation. Future research on myxozoans from large elasmobranch and teleost hosts inhabiting a range of different depths as well as on infections and spore types in deep-water invertebrate hosts could further contribute to an understanding of adaptive morphotypes and their distribution in different aquatic habitats, as well as to the evolutionary history of the Myxozoa.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ijpara.2021.05.008>.

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